# Using Hydroacoustics to Describe Pelagic Fish Distribution in the Penobscot Estuary, Main


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## Abstract

Temperate estuaries are inherently variable and productive ecosystems that provide nursery habitat, migration pathways, and forage areas for diadromous, estuarine, and marine fish. We used multifrequency scientific echo sounders (SIMRAD EK60 split-beam, 38 and 120 kHz) to describe the distribution of pelagic fish in the Penobscot River estuary, Maine, in 2012 and 2013. Differences in responses between frequencies were used to distinguish fish from other biota. Acoustic area backscatter from echo integration ($s_A$ [m$^2$/nautical mile$^2$], a common measure proportional to fish density) and target strength (TS; dB re 1 m$^2$, an acoustic measure of fish size) distributions varied with season and salinity. Overall, the $s_A$ and TS distributions were similar in both years, with detectable spatial and temporal patterns. The highest value of $s_A$ occurred in July of both years, when dense schools of fish were detected in higher-salinity areas of the lower estuary. The middle estuary had high $s_A$ values in April both years, particularly in the vicinity of the seawater–freshwater interface. The mixing area in the middle estuary stratum appears to be important fish habitat; we found fish in this area throughout the year. Fish of variable TS were using this mixing zone throughout the survey period. In full freshwater, upstream from the salinity mixing area, $s_A$ was generally low. The majority (~77%) of discrete fish detected had TS values less than −42 dB. The TS distributions varied seasonally, with the highest TS measurements occurring more frequently in April and May and the lowest ones occurring most frequently in July and August. This study demonstrates the efficacy of using a mobile hydroacoustic survey to assess pelagic fish distribution in a complex estuary and may provide a template for long-term monitoring in dynamic estuarine ecosystems.


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## Article

Temperate estuaries are complex habitats. River discharge, tide, and bathymetric features vary widely across and within individual estuaries; these features also interact in complex ways. On an annual basis, river discharge varies substantially and can determine the area and volume of estuarine habitat (Pritchard 1967). At a daily scale, river discharge and tide can interact to change environmental conditions from nearly entirely marine to freshwater at a single location (Prandle 1985). Thus, fish using estuaries are adapted to cope with constantly changing environmental conditions (Elliot et al. 2007). Many species use estuaries only during certain life stages (Able 2005), and tolerance for fluctuating salinity is greatest for resident estuarine species (Nordlie 2003). Despite the physical stresses present, temperate estuaries in the northeast United States are productive habitats (Jury et al. 1994; Roman et al. 2000). Many ecologically and commercially important fish species have developed various ways to exploit resources available in estuaries (Ray

2005); for example, many clupeid species use these highly productive habitats as nursery areas while gaining refuge from marine predators (Townsend et al. 1989; Stevenson and Scott 2005; Martinho et al.2012). Understanding fish habitat use in estuaries is challenging for a variety of reasons. First, the physical complexity of the estuarine environment itself makes traditional fish capture methods (e.g., trawls, trap nets, and seines) difficult to deploy (Livingston 1987). Second, many diadromous species are at historically low abundance levels across the North Atlantic basin (Limburg and Waldman 2009), which further exacerbates the challenges of understanding the dynamics of estuarine habitat use. In particular, some species that use north eastern U.S. estuaries are either threatened or endangered (e.g., pursuant to the U.S. Endangered Species Act), compelling a minimally impactful sampling approach (Use of Fishes in Research Committee 2014). Third, diadromous species use estuaries in different ways. Estuaries can be used as migratory corridors (e.g., Alewife *Alosa pseudoharengus* and Blueback Herring *A. aestivalis* [collectively referred to as river herring]: Fay et al. 1983; Atlantic Salmon *Salmo salar:* Kocik et al. 2009), as staging areas (e.g., American Shad *A. sapidissima*: Dodson et al. 1972; Atlantic Salmon: Randall et al. 1991), for overwintering (e.g., *Alosa spp*.: Street et al. 1975; Limburg1998), as juvenile nurseries (e.g., *Alosa spp*.: Ray 2005; Rainbow Smelt *Osmerusmordax*: Sirois and Dodson2000), and for reconditioning of post spawn adults (e.g., Atlantic Salmon: Moore et al. 1995). These uses vary by season, species, and life stage (Elliott et al. 2007; Able and Fahay 2010). Fourth, some marine species use estuaries as nursery areas or opportunistically for feeding (e.g., Atlantic Herring *Clupea harengus*: Townsend et al.1989). Lastly, because fish in estuaries have complex distribution and variable life history patterns particular attention to spatial scale is required when patterns of fish abundance and habitat use are described (Livingston 1987).

The constantly changing physical conditions in estuaries require robust approaches to fisheries monitoring. Active acoustics using scientific split-beam echo sounders can address some of these challenges and are increasingly being used to monitor fish distributions in estuaries (Boswell et al. 2007; Samedy et al. 2013). The application of mobile echo sounders in estuaries can provide information on pelagic fish distribution, density, and size over relatively large spatial scales (Stables et al. 2005; Goodbrand et al. 2013) and are particularly useful where fisheries data are sparse (Horne 2005). This approach offers the potential to estimate relative densities of pelagic species and determine fish size distributions in these poorly understood ecosystems (e.g., Guillard et al. 2004). In estuaries, where fish species diversity is generally low (Elliot and Quintino 2007), habitat tolerances (e.g., to salinity) and migration timing (e.g., diadromous fish) are seemingly predictable. Clupeid species, for instance, are known to exhibit schooling behavior from juvenile stages onwards (Gallego and Heath 1994; Martinho et al.2012), and when schooling species are present in the estuary school morphology and response differences of backscatter strength can be used as distinguishing features on acoustic echograms (Jech and Michaels 2006; Korneliussen et al. 2009). This prior knowledge can be used to develop an acoustic survey with realistic objectives. Other advantages of mobile acoustic surveys include cost-effectiveness while providing broad spatial coverage. Acoustic surveys are low impact and can be conducted in areas that are difficult to survey with other methods (e.g., Boswell et al. 2007). Indices of species' abundance and biomass from repeated acoustic surveys can provide a long-term monitoring method (Rudstam et al. 2009). We therefore suggest that these factors make mobile active acoustics suitable for assessing fish distributions in estuaries.

In the following sections, we describe changes in pelagic fish areal backscatter ($s_A$ = in units $m^2/nmi^2$ [nmi = nautical mile = 1.85 km]) and target strength (TS = in units dB re 1 $m^2$ [dB = decibel]) distributions over space and time in a poorly understood temperate estuary the Penobscot River estuary, Maine) using mobile multifrequency split-beam hydroacoustics. Our specific objectives were to monitor changes in (1) pelagic fish $s_A$ distributions and (2) pelagic fish TS distributions over both space and time.

# METHODS

*Study site.*—The Penobscot estuary is a drowned river estuary with a complex mixing regime that varies with freshwater flow and tidal height, which in turn influence temperature and salinity conditions (Haefner 1967). The Penobscot River is the second largest in New England and has an annual average discharge of 465$m^3$/s (USACE 1990). The Penobscot estuary has a tidal range of 3–4 m (NOAA–NOS 1985), providing habitat for many imperiled fish species (Saunders et al. 2006). The Penobscot River has a long history of fish passage barriers but is currently undergoing a significant restoration effort resulting in the removal or bypassing of three lower main-stem dams (Day 2006). These passage improvements should substantially improve the connectivity between freshwater and marine systems for many diadromous species (Trinko Lake et al. 2012), and their long term effects may be measurable in the estuary. Our survey area extended from Bangor to Fort Point, Stockton Springs, Maine (Figure 1). We divided the area into upper, middle, and lower estuary strata based on generally observed salinity ranges (between 0 and 35‰; sensu Haefner 1967). The upper stratum is heavily influenced by the Penobscot River and is predominantly freshwaterwithamaximumdepthof~15m.The middle estuary is where freshwater and seawater mix, which often produces a “salt wedge” that can result in distinct salinity layers. This stratum has variable salinity (5–20‰) and a maximum depth of ~20 m. The lower stratum is strongly influenced by Penobscot Bay; salinity is variable (20–35‰) and maximum depth is ~35 m. The environmental conditions within this system are widely variable and dynamic; however, the strata we developed here are generally reflective of these conditions.

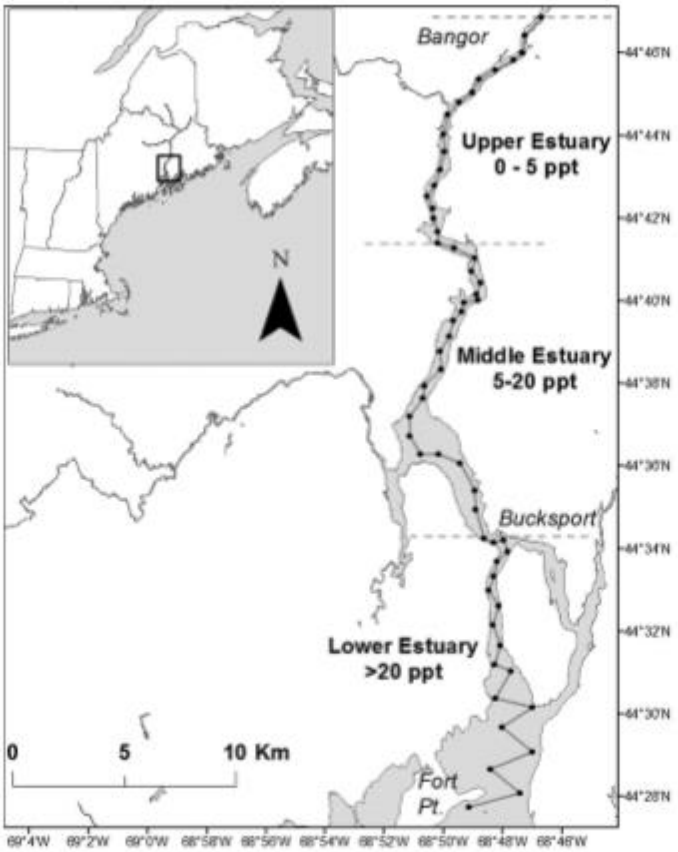


FIGURE 1. The Penobscot estuary, Maine, with the three predefined survey strata (upper, middle, and lower estuaries) based on approximate surface salinities (ppt – ‰). The acoustic survey transects with waypoints are also shown (solid gray line and black dots).

*Survey design.*—We implemented a systematic survey design along predetermined zigzag transects by establishing waypoints on both sides of the estuary in water no less than 6 m depth (Figure 1). This design is considered the most appropriate for narrow estuarine–riverine channels (Simmonds and MacLennan 2005). Each straight-line path between waypoints was considered a single transect in postprocessing. The region of interest (area of estuary > 6 m depth where transects occurred) had an area of approximately 16 $km^2$ (upper estuary, 3.25 $km^2$ middle estuary, 4.5 $km^2$; lower estuary, 8.25 $km^2$; Figure 1), with a total transect length of approximately 42 km. This resulted in a degree of coverage of 10.5 (total transect length /√total survey area: Aglen 1989). At an average speed of 8 km/h, each survey took approximately 5 h to complete. Transects were completed during daylight and in the direction of tidal flow; therefore, transect direction (upstream or downstream) varied depending on tidal cycle. The end and start points of each survey (depending on direction of travel) were generally at high tide in the upper stratum and generally at low tide in the lower stratum. Therefore, the middle stratum was always surveyed at midtide during the ebb or flood stages. We recorded salinity data at 1-min intervals with a YSI datasonde 6920 (YSI, Yellow Springs, Ohio), which was attached to the transducer frame 0.50 m below the water surface.

We used portable split-beam scientific echo sounders (SIMRAD EK60 general purpose transceivers: Andersen 2001) with 38 kHz (circular 12°) and 120 kHz (circular 7°) operating frequencies. The transducers were frame-mounted from the port side of the 6.1-m-long, aluminum, skiff survey boat. Transducer faces were 0.50 m below the surface of the water and 0.35 m apart (center to center). We used a laptop computer with an internal GPS receiver to obtain location information for the acoustic files. The system was powered by a deep-cycle, 12-V, DC battery independent of vessel electronics. Echo-sounder parameters for both frequencies were as follows: 0.256-ms pulse duration, 4-Hz ping rate, and 500-W transmission power. Standard calibrations (using the SIMRAD LOBE program) were done monthly in slack tide conditions in the lower estuary (salinity, ~35‰) by using a 38.1-mm, tungsten carbide, standard target sphere (Foote et al. 1987; SIMRAD 2003). The sphere was suspended 10–15 m below the transducers during calibrations. Temperature and salinity of ambient conditions were used during echo-sounder calibrations. The median temperature and salinity for the entire range of the survey was used in postprocessing the acoustic data.

*Acoustic data processing*.—We analyzed data from 0.10 m above the seabed and 3.00 m below the surface to avoid unwanted echo returns from the bottom and from bubble interference at the surface. We used Echoview software (version 5.4; Myriax Pty Ltd, Hobart, Australia; http://www. myriax.com) to process the acoustic data. We scrutinized echograms with the mean volume backscattering strength ($s_v = 10.\log_{10}(s_v)$ [dB re 1 $m^{-1}$]: MacLennan et al. 2002) threshold at −70 dB to filter weak backscatter and aid in scrutiny (ICES 2015a, 2015b). Data were synchronized by time between frequencies. We applied a 3 × 3 convolution matrix (with summation of the kernel coefficients = 1) to the 38-kHz and 120-kHz $s_v$ data to smoothen the strongest backscatter (Jech 2014).

The data-processing technique of dB differencing was applied to the $s_v$ data, exploiting the frequency-specific response to classify categories of scattering types (Simmonds and MacLennan 2005; McKelvey and Wilson 2006). Species identification with single frequency narrowband acoustic techniques is problematic (Stanton et al. 2010); however, using multifrequency analysis can improve the classification of backscatter (Korneliussen et al. 2009). These techniques rely on the frequency response of each species to help discriminate fish with swim bladders (Jech and Michaels 2006; McKelvey and Wilson 2006; DeRobertis et al. 2010) from other types of scatterers (e.g., euphausiids, fish without swim bladders), making broad size-based categorization possible (Madureira et al. 1993; Korneliussen and Ona 2003). Multifrequency approaches are currently used to survey many pelagic fish species over large spatial scales, particularly in the marine environment (Fernandes et al. 2002). Analysis regions with differences in the frequency response ($s_v$ [120] − $s_v$ [38]) < 10 dB were classified as fish, while regions with differences > 10 dB were excluded from the fish analysis and were classified as other biota (Jech 2014). The acoustic area backscattering coefficient, $s_A$ ($s_A$ =4 π(1852)²$s_a$, where $s_a$ is in units of m²/m² and is the integral of $s_v$ over a finite range), is an areal representation of all integrated backscatter in the beam (Simmonds and MacLennan 2005). We vertically integrated the water column $s_v$ classified as fish and horizontally averaged an entire transect to obtain the $s_A$ for each transect. We interpreted$s_A$ as a measure of areal fish density.

We used the Fish Schools Detection module in Echoview to describe dense fish schooling behavior in terms of space and time. The acoustic density of schools depends on many factors, including size, species and even tilt angle of fish in the school (Simmonds and MacLennan 2005). The presence of areas of strong backscatter within a fish school (e.g., $s_v$ ≥−30 dB) coupled with a multifrequency comparative approach can be used with fish capture methods to establish the species present in the school (Stables et al. 2005; Jech and Michaels 2006; Korneliussen et al. 2009). Backscatter from fish in close proximity (e.g., schooling) is difficult to attribute to individual fish and not suitable for single target size analysis (described below). The combined backscatter from all fish in a school is proportional to the overall fish density in the school. Therefore, fish in schools were included in the overall integrated backscatter estimates for each survey, but the backscatter from schools was excluded in the analysis of target strength from individual fish. Fish school detection settings were as follows: minimum total school length = 2.00 m, minimum total school height = 1.50 m, minimum candidate length = 2.00 m, minimum candidate height = 1.50 m, maximum vertical linking distance = 1.00 m, maximum horizontal linking distance = 2.00 m, and distance mode = GPS distance. Fish in aggregations not meeting the above criteria were not considered to be in schools for this study.

The target strength (TS $= 10.\log_{10}[\sigma_{bs}]$, in units dB re 1 m², where $\sigma_{bs}$ is the backscattering cross section [MacLennan et al. 2002]) of discrete targets gives an indication of the size of the organism, although the translation of TS to length has a stochastic component and can be biased due to a number of factors including the target tilt angle, depth, and material properties (Fässler et al. 2009). We used the single-target detection algorithm in Echoview with the parameter settings: pulse-length determination level, 6 dB; minimum normalized pulse length, 0.25; maximum normalized pulse length, 1.5; and maximum beam compensation, 12 dB. We used the Fish Tracking Module in Echoview to identify and categorize single or multiple consecutive targets as discrete individual fish derived from the 38-kHz single target detections echogram. Single target detections from within schools were not considered suitable for fish tracking analysis and were therefore excluded. We selected the maximum TS when there were multiple target detections for the same fish. Fish track detection settings were: TS threshold = −55 dB, minimum number of single targets in a track = 1, and maximum gap between single targets = 0. We used the −55 dB threshold to filter out weak backscatter to focus on postlarval fish responses only; the target strength of many of the main species inhabiting the estuary (e.g., Saunders et al. 2006) have been estimated empirically (e.g., Foote 1987; Rudstam et al. 2003; Simmonds and MacLennan 2005; Gurshin 2012).

# RESULTS

## Fish Distribution Patterns

We found different generalized aggregating behaviors of fish throughout the estuary from echogram scrutiny. While the overall distribution in terms of acoustic density was spatially patchy, within areas of higher density fish appeared to aggregate in similar ways. Discrete fish targets were detected in the upper and middle estuary transition area in April in both years in low (surface) salinity, generally in the bottom half of the water column (Figure 2a). Fish were generally well dispersed throughout the water column in the middle estuary in May throughout both years (Figure 2b). Fish frequently aggregated in layers, as seen in November 2012 in the lower estuary (Figure 2c). Fish aggregated in dense schools (e.g., $s_v$ mean for the school≥−30dB) from June through August in higher (surface) salinity (>20‰) areas of the lower estuary (Figure 2d). Schools were entirely absent in the survey area in April of 2012 and 2013 (Table 1) and from the upper estuary during the entire survey period. In the middle estuary, fish schools were detected in June, July, and August of 2012 and only in August in 2013. In the lower estuary, the first evidence of fish schools was in May in both 2012 and 2013, and schools were present through to November.

TABLE 1. Acoustic transect and freshwater flow data for 2012 and 2013 in the Penobscot estuary, Maine. The mean $s_A$ values for the upper, middle, and lower estuaries are shown for each stratum (SDs in parentheses). The survey direction is represented by ↑ (upstream) and ↓ (downstream). The average daily discharge flow (m$^3$/s) was reported from river kilometer 53 at U.S. Geological Survey gauging station 01034500 in 2013 (available: http://waterdata.usgs.gov gauge number 01034500); Y = yes, N = no.

| | | Mean $s_A$ (m$^2$/nmi$^2$) | | | Presence of fish schools | | | |
|---|---|---|---|---|---|---|---|---|
| Date | Flow (m$^3$/s) | Upper | Middle | Lower | Upper | Middle | Lower | Survey direction |
| **2012** | | | | | | | | |
| Apr 11 | 332 | 75 (142) | 888 (752) | 54 (40) | N | N | N | ↑ |
| Apr 26 | 1,178 | 11 (7) | 115 (154) | 442 (484) | N | N | N | ↑ |
| May 3 | 628 | 54 (110) | 284 (237) | 96 (153) | N | N | N | ↓ |
| May 10 | 470 | 79 (66) | 290 (188) | 72 (56) | N | N | Y | ↑ |
| Jun 19 | 217 | 131 (91) | 151 (97) | 583 (405) | N | Y | Y | ↑ |
| Jul 25 | 151 | 55 (54) | 151 (156) | 1,496 (843) | N | Y | Y | ↑ |
| Aug 15 | 131 | 35 (31) | 106 (119) | 568 (429) | N | Y | Y | ↑ |
| Nov 20 | 379 | 4 (3) | 161 (91) | 264 (328) | N | N | Y | ↑ |
| **2013** | | | | | | | | |
| Apr 15 | 561 | 13 (20) | 704 (674) | 44 (73) | N | N | N | ↑ |
| Apr 26 | 841 | 15 (23) | 212 (225) | 72 (76) | N | N | N | ↑ |
| May 2 | 504 | 10 (14) | 253 (200) | 66 (56) | N | N | Y | ↓ |
| May 21 | 337 | 152 (114) | 201 (108) | 88 (96) | N | N | Y | ↓ |
| Jun 13 | 1,314 | 12 (7) | 98 (51) | 234 (141) | N | N | Y | ↑ |
| Jul 25 | 392 | 52 (18) | 192 (125) | 1,040 (937) | N | N | Y | ↑ |
| Aug 29 | 224 | 76 (49) | 242 (368) | 186 (141) | N | Y | Y | ↓ |
| Nov 15 | 271 | 66 (78) | 126 (66) | 149 (71) | N | N | Y | ↑ |

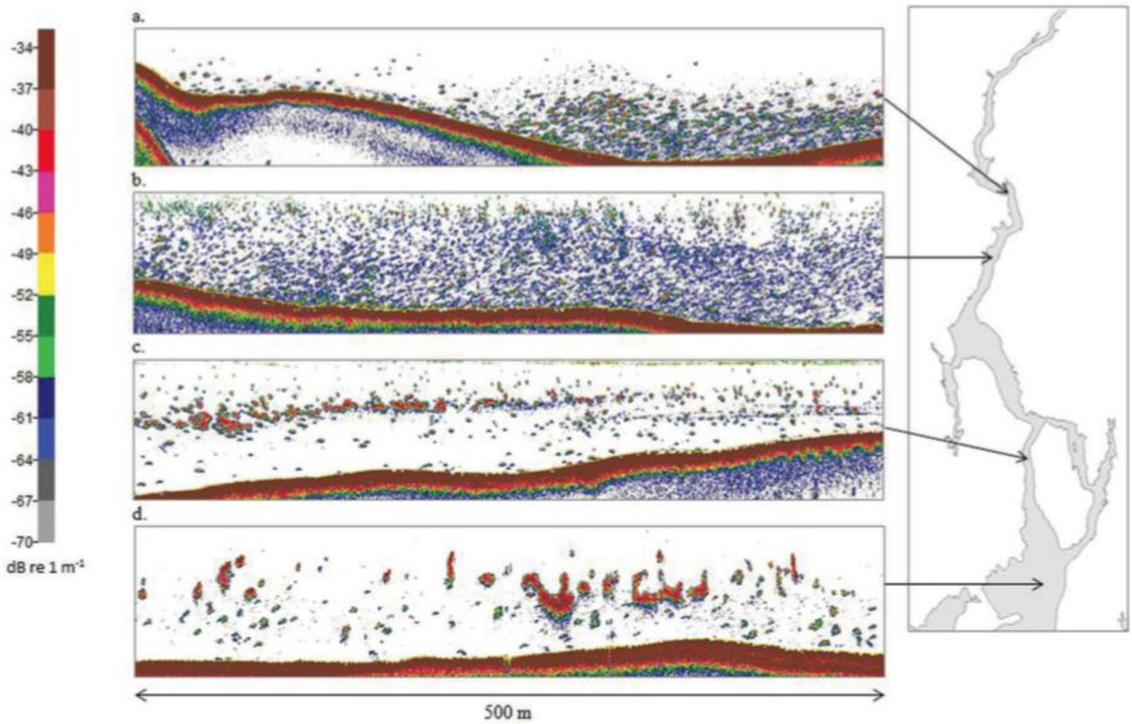


FIGURE 2. Example echograms of 38-kHz $S_v$ categorized as fish from dB differencing with a −70-dB threshold, showing different fish aggregating behavior: (**a**) individual fish aggregating in the lower half of the water column in low salinity in the middle estuary in April, (**b**) fish aggregating more homogenously throughout the water column, with some evidence of layering in the middle estuary in May, (**c**) distinct layers of individual and schooling fish midwater in the lower estuary in November, and (**d**) a high density of pelagic schooling fish in the lower estuary in July. The scale bar relates to backscatter intensity.

## Fish Densities

Acoustic $s_A$ (fish density) of transects varied over space and time during the study period, but the overall spatial patterns were similar in 2012 and 2013 (Figures 3–6). Spatially, neighboring transects tended to have similar $s_A$ (Figures 3, 4), but at the stratum scale, fish aggregated in spatially heterogeneous patterns with generally high standard deviations of estimates between transects within each stratum. The $s_A$ of transects in the lower estuary were the most variable of the three strata with some of the highest (~1,000–1,500 $m^2/nmi^2$) and lowest values recorded. The largest overall mean $s_A$ occurred in July 2012 in the lower estuary (Table 1); this coincided with an increase in fish schools detected in the lower estuary during that time. There was similarly high mean $s_A$ in July 2013. Conversely, the lower estuary had relatively low mean $s_A$ in April and May in both years, apart from April 26, 2012, which coincided with high freshwater flow into the estuary. Typical values for mean $s_A$ in the middle estuary were in the region of 100–300 $m^2/nmi^2$ (Table 1); however, the highest estimates of mean $s_A$ in the middle estuary occurred in early spring (April) on the first survey in both years (~700–900 $m^2/nmi^2$). The upper estuary generally had relatively low mean $s_A$ (<100 $m^2/nmi^2$); but there were some increased values observed (e.g., June 19, 2012, and May 21, 2013). Both instances occurred just upstream from the mixing zone (Figures 5, 6). Fish distributions seemingly varied with salinity, causing areas of high and low $s_A$ (Figures 3–6). For example, during high flow conditions (~1,200m $^3$/s) on April 26, 2012 (Table 1), the salinity transition zone (~5‰) was farther downstream than on the subsequent survey on May 3, 2012 (flow, ~600 m3/s); similarly, the area of high $s_A$ at the transition zone was also farther downstream during these high flow conditions (Figures 3, 5).

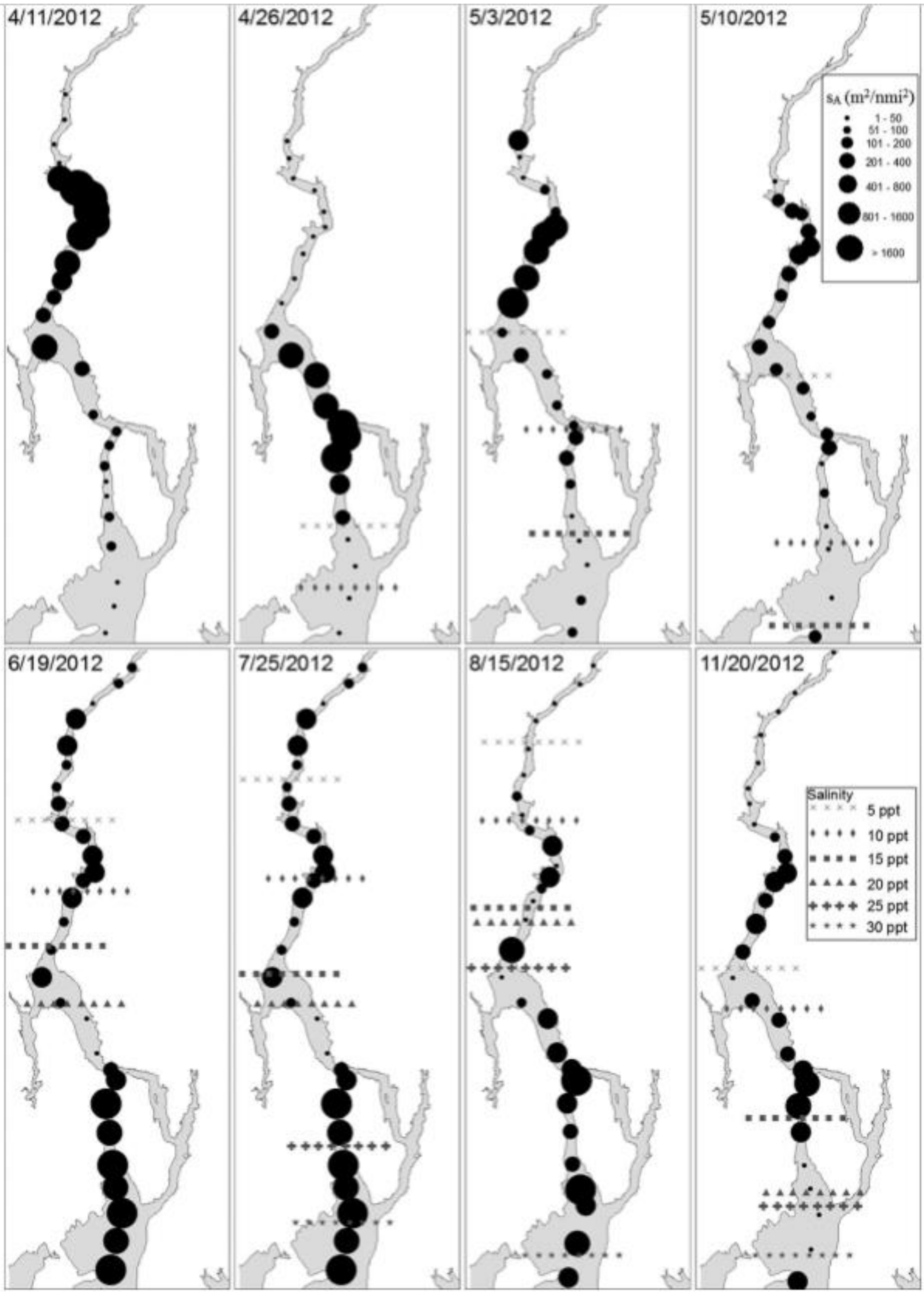


FIGURE 3. Spatial and temporal representations of $s_A$ ($m^2/nmi^2$) per transect from the integration of 38-kHz $S_v$ data categorized as fish and surface salinity in 2012 (only alternate transects are shown for clarity). Surface salinity (ppt – ‰) is displayed as an interval, with each horizontal symbol line corresponding to the downstream extent of that interval. Salinity data for April 11, 2012, were unavailable.

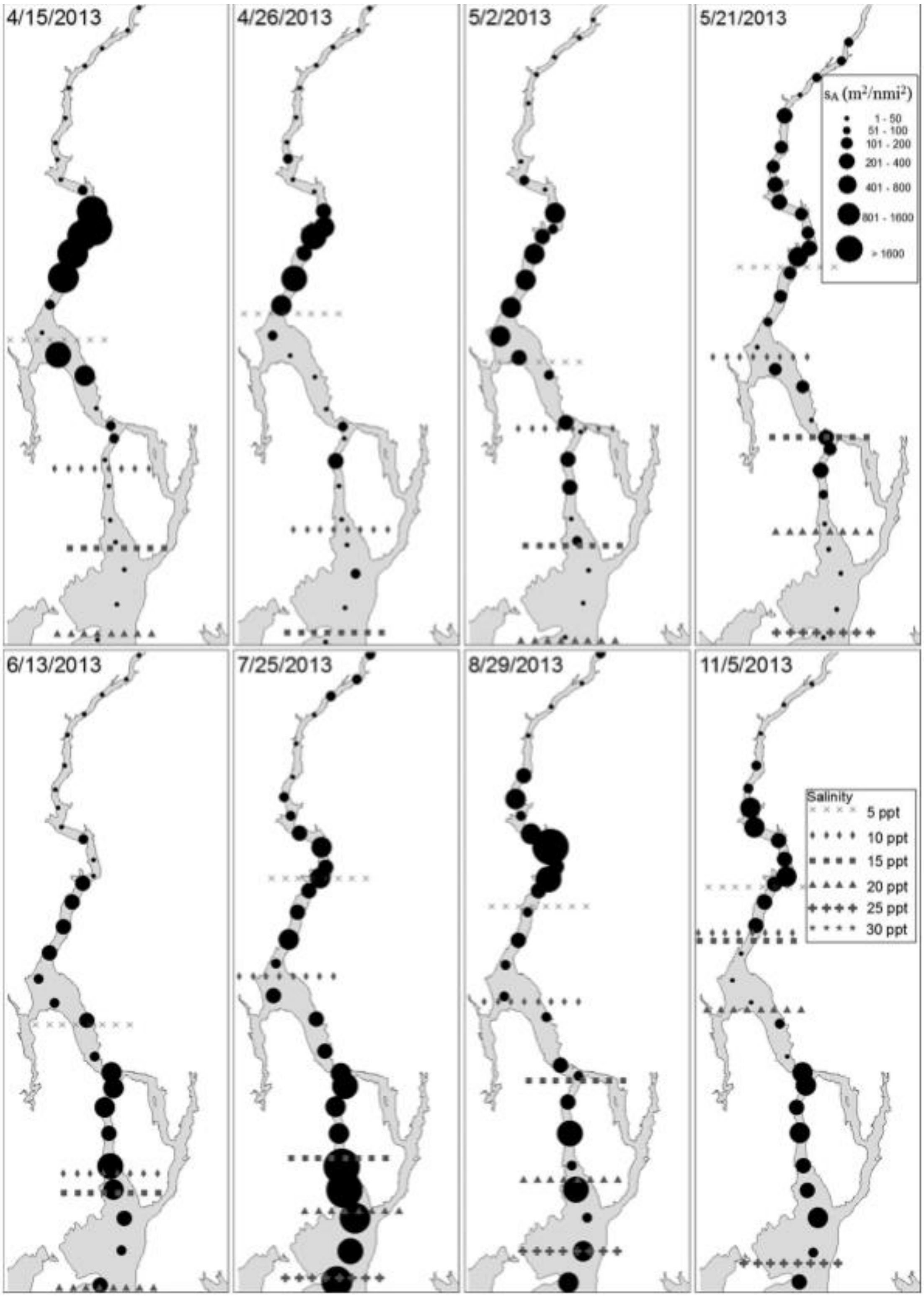


FIGURE 4. Spatial and temporal representations of $s_A$ ($m^2/nmi^2$) per transect from the integration of 38-kHz $S_v$ data categorized as fish and surface salinity in 2013 (only alternate transects are shown for clarity). Surface salinity (ppt – ‰) is displayed as an interval, with each horizontal symbol line corresponding to the downstream extent of that interval.

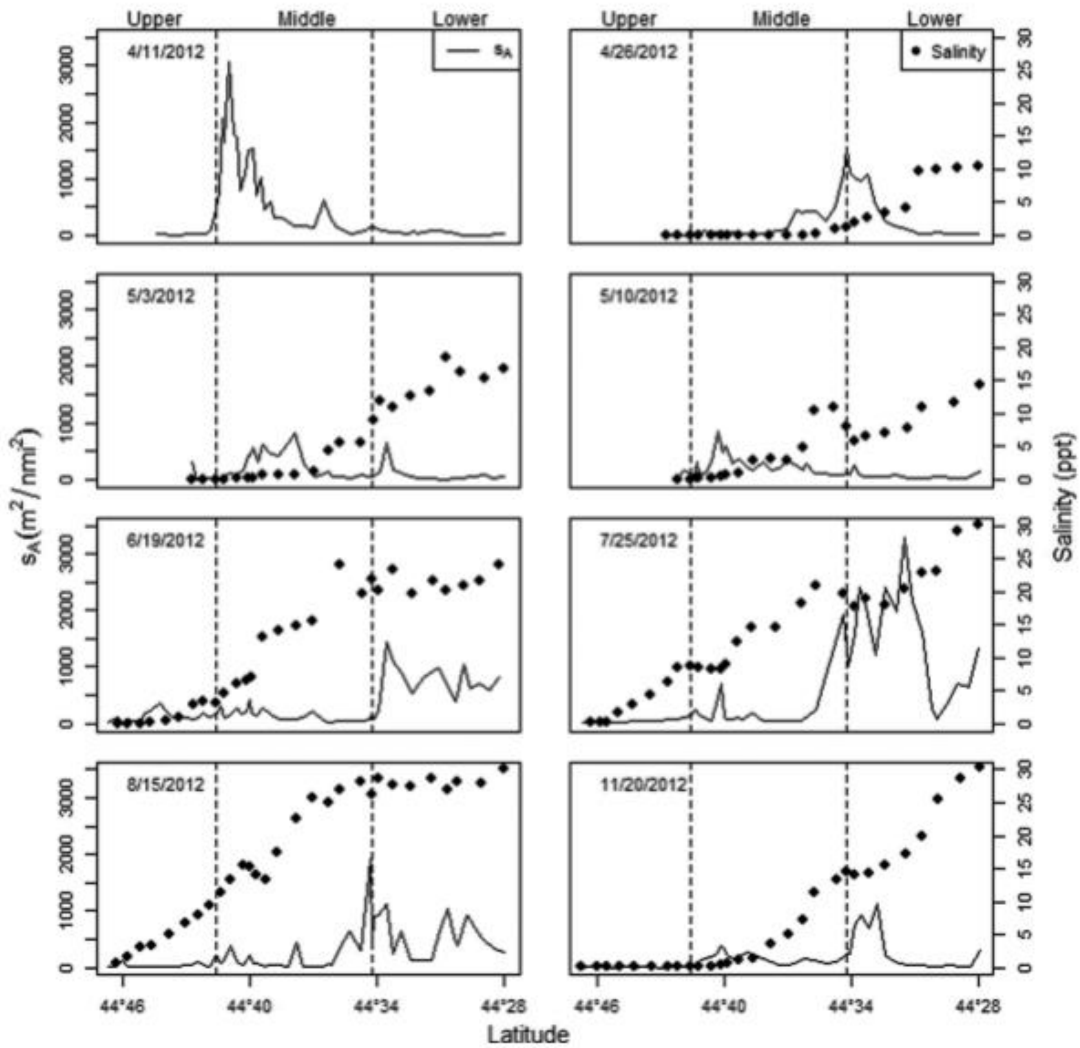


FIGURE 5. Estimates of $s_A$ from the integration of 38-kHz Sv data categorized as fish from dB differencing (left scale) and midpoint surface salinity (ppt = ‰) per transect (right scale) as a function of latitude in 2012. The vertical dashed lines are the locations of the approximate boundaries between the three defined strata (upper, middle, and lower estuaries). Salinity data for April 11, 2012, were unavailable.

## Salinity

Surface salinity (including the location of the mixing zone) was variable throughout the surveys in both years. We defined the geographical middle estuary (Figure 1) based on expected salinities in the region of 5–20‰ from Haefner (1967), and this stratum generally encompassed this salinity transition (Figures 5, 6). High $s_A$ values were measured upstream from the surface salinity mixing area in both years in April. Likewise, the high $s_A$ observed when schools were present in the lower estuary in July in both years coincided with high surface salinity throughout the lower estuary during this time.

## Fish TS Distributions

The majority (~77%) of individual fish targets detected overall in the estuary had TS < −42 dB (Figures 7, 8). We identified a temporal pattern in the distribution of fish TS during both years, with TS > −42 dB occurring more frequently in April and May (Figures 7–9). From the cumulative frequency graphs in Figure 9, higher-TS fish were more numerous in April in the upper and middle estuaries and fish with a lower TS were more prevalent in July. By November, slightly higher TS fish were present in the upper and middle estuaries compared with July. There was an increase in fish tracks with TS < −42 dB (Figure 7) in the lower estuary during July–November 2012 (Table 1). There was also some evidence that lower-TS fish were more common in the lower estuary in April, and there was an increase in higher-TS fish in May and June, particularly in 2013 (Figure 9).

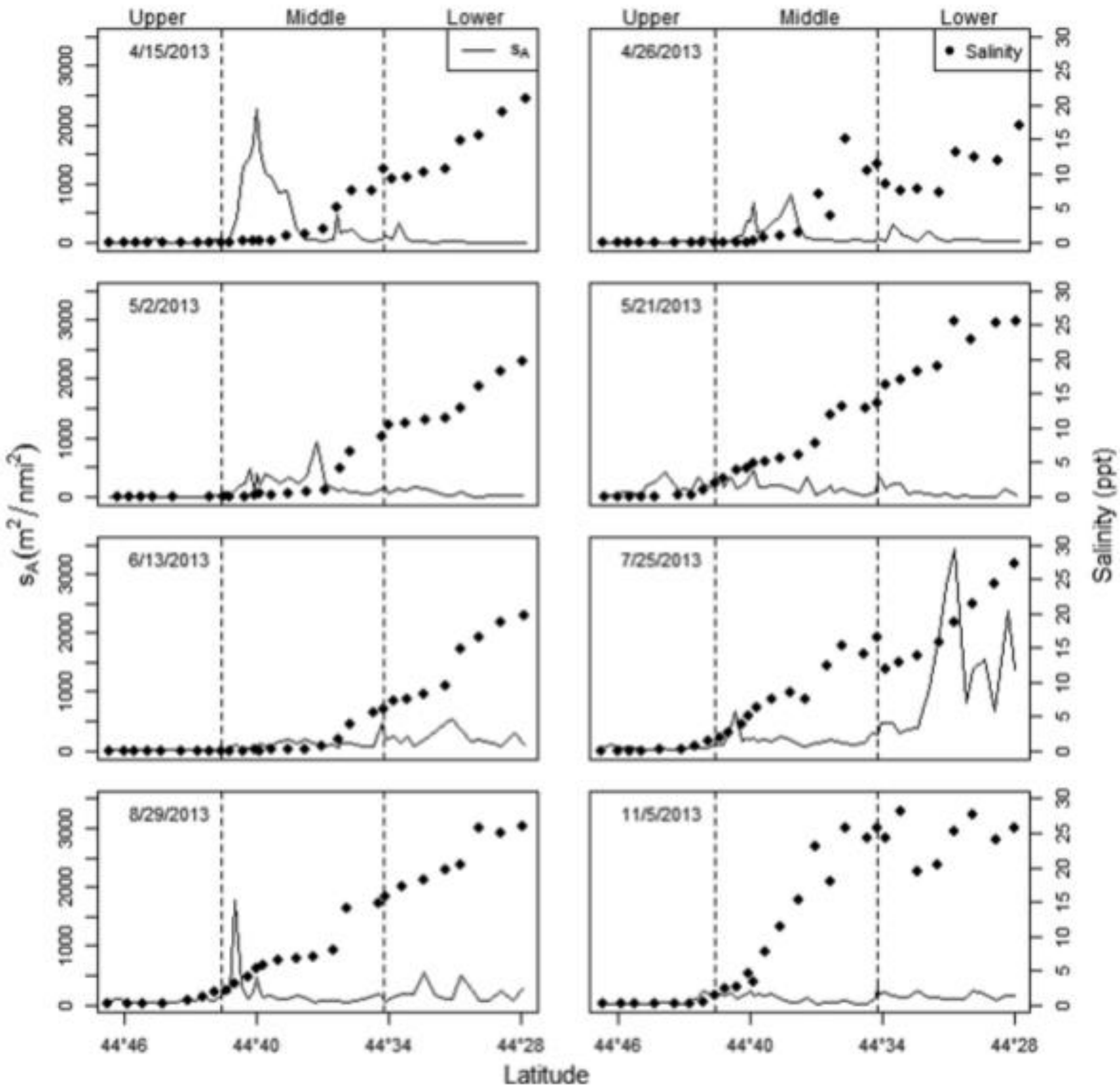


FIGURE 6. Estimates of $s_A$ from the integration of 38-kHz $S_v$ data categorized as fish from dB differencing (left scale) and midpoint surface salinity (ppt = ‰) per transect (right scale) as a function of latitude in 2013. The vertical dashed lines show the approximate locations of the boundaries between the three defined strata (upper, middle, and lower estuaries).

## DISCUSSION

We described fish aggregation and distribution patterns using acoustic fish density ($s_A$), acoustic size (TS) frequency distributions, and surface salinity levels in the Penobscot estuary between April and November in 2012 and 2013. We were able to monitor changes in pelagic fish over space and time in both $s_A$ distributions and TS distributions. The methods developed here provide a template for monitoring pelagic fish in the Penobscot estuary and similar temperate estuaries.

In the upper estuary, $s_A$ was somewhat variable throughout the survey period, but mean $s_A$ was typically lower than that in both the middle and lower estuaries. We expected to find evidence of diadromous fish using the upper estuary as a migration corridor, particularly from April through June. Our inability to detect a substantial increase in $s_A$ in the upper estuary during this time could be attributable to the low overall diadromous fish density in the Penobscot river–estuary system, insufficient survey coverage (either spatially or temporally), or a combination of these factors. The degree of coverage we achieved was acceptable for such acoustic surveys (Simmonds and MacLennan 2005), but this design does not deal with any habitat outside of our strata boundaries. The survey design included areas with relatively shallow water, but the distribution of fish outside the survey area and therefore not covered by the survey is unknown. Many of the species expected to inhabit the estuary do so for a specific period, including migrating diadromous species. Diadromous species (e.g., *Alosa spp*.) have generally predictable migrations and are expected to be susceptible to this survey design provided surveys are done with a frequency that captures the period during their migration through the system. An adequate frequency to conduct an acoustic survey in this system to capture diadromous migrations is as yet unknown. Fish may be missed if they inhabit areas outside of the survey area (e.g., when overwintering and/or staging) or if diadromous migrations occur through the estuary over a particularly condensed period between successive surveys. There was some evidence of slightly increased $s_A$ (and relatively low TS) in the upper estuary on June 19, 2012 (mean $s_A$ ≈ 131 $m^2/nm^2$, TS mode ≈−48 dB) and May 21, 2013 (mean $s_A$ ≈ 152 $m^2/nm^2$, TS mode ≈−42 dB), but it is not clear whether this increase was due to higher densities of diadromous fish. The increases in $s_A$ observed in these instances were just upstream from the salinity mixing zone, which is itself physically dynamic. This distinction between the strata is often blurred, and therefore the presence of fish upstream from the mixing zone in low salinity may indicate the presence of diadromous or freshwater fish. Regardless, detecting an increase in $s_A$ in the upper estuary during this time is encouraging and suggests that monitoring diadromous fish presence in the freshwater part of the estuary is a reasonable objective in the future. The high variability in $s_A$ estimates between transects within this stratum during this time suggests that fish were patchily distributed. Also, while the Penobscot estuary is clearly a migratory corridor for diadromous fish (Saunders et al. 2006), we expected the upper estuary would be readily used by freshwater species such as Smallmouth Bass *Micropterus dolomieu*, White Perch *Morone americana*, and Yellow Perch *Perca flavescens*. Our results suggest that their abundance is either relatively low in this area or they occupy near-shore or demersal habitat not sampled in the survey, or both.

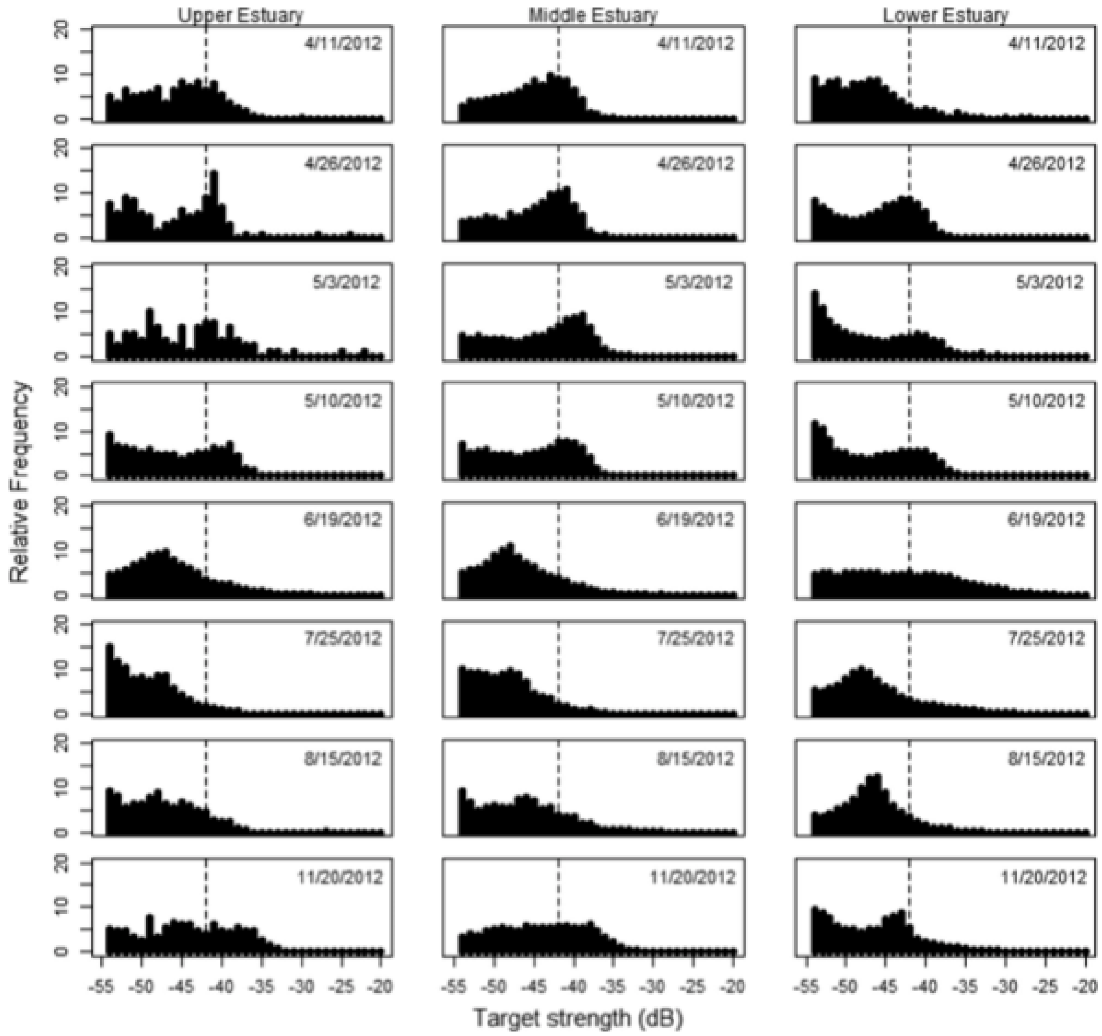


FIGURE 7. Relative frequency distributions of fish tracks by target strength (maximum TS per fish track [38 kHz]) detected in the upper, middle, and lower estuaries from transects conducted in 2012 (fish in schools are not included). The vertical dashed lines at −42 dB correspond to approximately 30 cm TL for physostomes (Foote 1987).

The middle stratum appears to be important fish habitat; we found moderate levels of pelagic fish $s_A$present in this area throughout the survey, particularly in the vicinity of the freshwater–seawater interface. The exception was in April, when we observed a much higher $s_A$ in this area in both years. The increased $s_A$ observed in the middle estuary during April is interesting in the context of diadromous fish migration; it is reasonable to expect some diadromous fish to stage during migration through the estuary (e.g., American Shad: Dodson et al. 1972; Atlantic Salmon: Randall et al. 1991) and that their preferred habitat would be somewhat driven by localized salinity conditions as they adjust to the changing salinity environment. Fish were apparently in the middle estuary before the first survey (April) in both years, suggesting overwintering (e.g., river herring: Street et al. 1975; Limburg 1998) or prespawning aggregations (e.g., Rainbow Smelt: McKenzie 1964). The increase in $s_A$ also coincided with fish of generally higher TS in the middle estuary during this time, suggesting the presence of strong scatterers at 38 kHz. If diadromous fish use the middle estuary to stage before migrating into full freshwater, this would explain the increased $s_A$ in April (Table 1) and higher modal TS in the middle estuary in April through June (Figures 7, 8). The increase in TS during this time is clearer in 2013, but trends were similar in both years. The extent to which diadromous species use the Penobscot estuary mixing area to stage as part of spawning migrations is currently unknown at this time. It also appears that fish of variable TS were using this mixing zone in the middle estuary throughout the survey period, potentially using the high turbidity, high productivity, and reduced salinity conditions to their advantage.

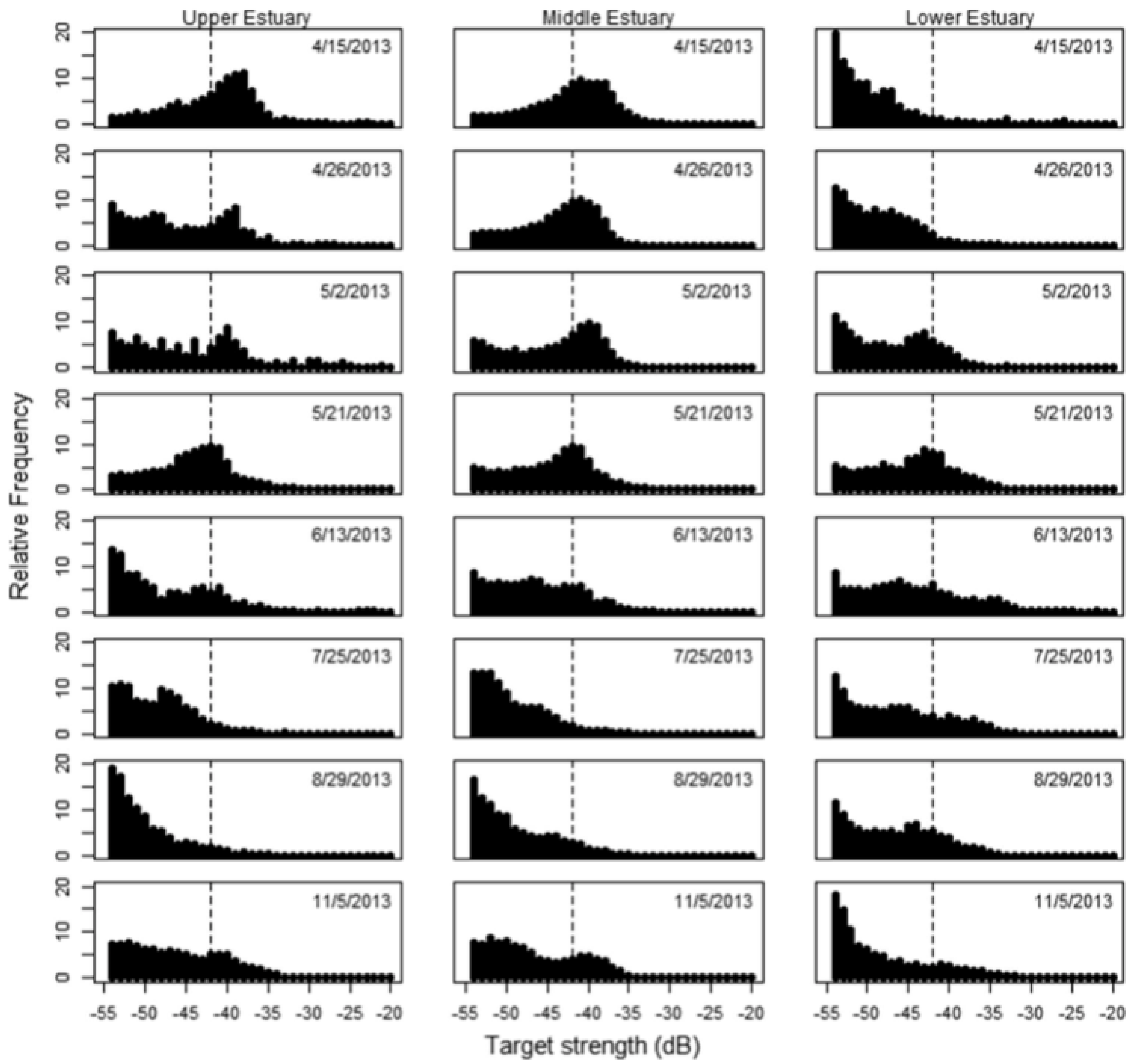


FIGURE 8. Relative frequency distributions of fish tracks by target strength (maximum TS per fish track [38 kHz]) detected in the upper, middle, and lower estuaries from transects conducted in 2013 (fish in schools are not included). The vertical dashed lines at −42 dB correspond to approximately 30 cm TL for physostomes (Foote 1987).

The precise location of the mixing area (as indicated from surface salinity measurements) in the middle estuary varied with tide and freshwater flow conditions, and the location of areas with high $s_A$ seemingly varied with the location of the mixing area. These results suggest that the choosing of strata is complex and the physical changes in the system, particularly with reference to salinity, need to be considered during survey planning. It may be that strata delineations based on general expected salinity conditions is not appropriate. For instance, on April 11, 2012, freshwater flow was low (~300 m³/s) and highest $s_A$ values were found in the middle estuary (Table 1).

On April 26, 2012, freshwater flow was very high (~1,200 m3/s) and highest $s_A$ values were roughly 10 km farther downstream. By May 3, 2012, freshwater flow dropped to 600 $m^3/s$ and the area of high $s_A$ values seemingly shifted back upstream. Fish distribution can alter with changes in hydraulic conditions in estuaries (Turek et al. 1987), and presumably this can be passive or active, depending on species and life stage. The salinity conditions are constantly influenced by tide and freshwater flow in the estuary, and therefore their influence on fish distribution is difficult to predict. The salinity profile throughout the water column is expected to be different from the surface, and a spatially offset lag occurs between surface and bottom salinities as these varying water densities mix. The formation of haloclines is also likely in some areas (Haefner 1967). Understanding the broad mechanisms driving fish distribution was informed with the surface salinity data we recorded; however, full depth salinity data are necessary to more accurately describe the distribution of fish in relation to environmental conditions. Surface salinity data generally revealed broad areas where mixing was occurring in the estuary, but the exact location and extent of the mixing area and occurrence of a salt wedge is not yet quantified.

The generalized equation derived by Foote (1987) for physostomes (fish that regulate gas in the swim bladder via a pneumatic duct connected to the esophagus, e.g., clupeids) at 38 kHz relates the TS to fish TL:

$$\mathrm{TS(dB)} = 20 \cdot \log L - 71.9,$$

where *L* is fish TL in centimeters. Based on this general relationship, a TS of −42 dB approximately equates to fish of 30 cm TL. Using a TS of −42 dB as a subjective size distinction between higher TS (large fish > 30 cm TL; e.g., migrating American Shad and Atlantic Salmon) and lower TS (small fish < 30 cm TL; e.g., river herring and Rainbow Smelt) pelagic fish, the study area consisted mainly of small fish (TS < −42 dB [i.e.,TL < 30 cm]). The choice of 30 cm TL as a distinction between large and small fish is a general guide, but is based on expected species present in the Penobscot estuary (Saunders et al. 2006) and the expected size range of these species (Collette and Klein-MacPhee 2002; Sheehan et al. 2011). The predominance of fish with lower TS values in the Penobscot estuary (77% of targets < −42 dB) is also similar to observations from a shallow estuary in Louisiana where Boswell et al. (2007) attributed roughly 70% of acoustic backscatter to targets < −47 dB. The TS range of fish targets we observed in April and May falls within predicted TL size ranges of species expected to inhabit north eastern temperate estuaries during this time (e.g., adult and subadult river herring: Saunders et al. 2006; Rainbow Smelt: Kovach et al. 2013; Atlantic Tomcod *Microgadus tomcod*: McQuinn and Nellis 2007). There was a seasonal component to the TS of fish found in the middle estuary early in the survey period (particularly April through June), and ~30% of fish detected were within the expected TS range (−46 to −42 dB [i.e. TL = 20–30 cm]) of some species of adult diadromous fish. Given the low salinity levels at the surface (typically less than 5‰) we can infer that the fish using this habitat must be euryhaline fish such as (but not limited to) river herring, Atlantic Tomcod, or Rainbow Smelt. The TS range (<−42 dB) also suggests that many of the fish targets we detected in the middle estuary throughout the year were young of year fish potentially using this area as a nursery (e.g., Rudstam et al. 2003; Gurshin 2012). Diadromous fish using northeastern U.S. estuaries for juvenile rearing has been noted in other systems (Grabe 1996) and is also likely to be important in the Penobscot estuary (e.g., MDMR and MDIFW 2009).

The temporal difference in distribution between surveys depending on season and the observed variability in TS is expected when the difference is caused by an influx of diadromous fish into the system. Overall, these results support the expectation that the upstream migration of adult diadromous fish causes a detectable increase in TS in the spring. The difference in TS distributions between early and late surveys is also likely to be influenced by the subsequent out-migration of juvenile diadromous fish, and more variability in TS results from the increased numbers of smaller fish. Many fish species are also known to use estuaries as nursery areas and as refuge from predators while benefiting from improved foraging opportunities (e.g., Elliot and Quintino 2007), further influencing the TS towards lower and more variable distributions.

The seasonal abundance of schooling fish in the lower estuary in July (with surface salinities of 20–35‰) is consistent with marine species' usage of this area, most likely for juvenile rearing. The $s_A$ was high in July particularly in the lower estuary, and the majority of fish were in dense schools during this time. These schooling fish in the lower estuary were most likely juvenile Atlantic Herring, as they are known to exhibit strong schooling behavior and use estuaries throughout their range during juvenile life stages (Townsend et al.

1989; Maes et al. 2005). Atlantic Herring are known to inhabit the outer Penobscot Bay in spring (e.g., Sheehan et al. 2011), and it is likely that they are also using the estuary in the summer when salinity is relatively high during reduced freshwater flow. Marine species that can also exhibit schooling behavior and are found in the lower Penobscot estuary include Atlantic Mackerel *Scomber scombrus* and sand lances *Ammodytes spp*.; however, their lack of a swim bladder makes it unlikely that they were included in our multifrequency analysis. These species will be detected, particularly with higher frequencies (e.g., 120 kHz); however, the decibel differencing criteria and the single target detection parameters we used would have most likely filtered backscatter from species without swim bladders during the analysis (e.g., Korneliussen et al. 2009).

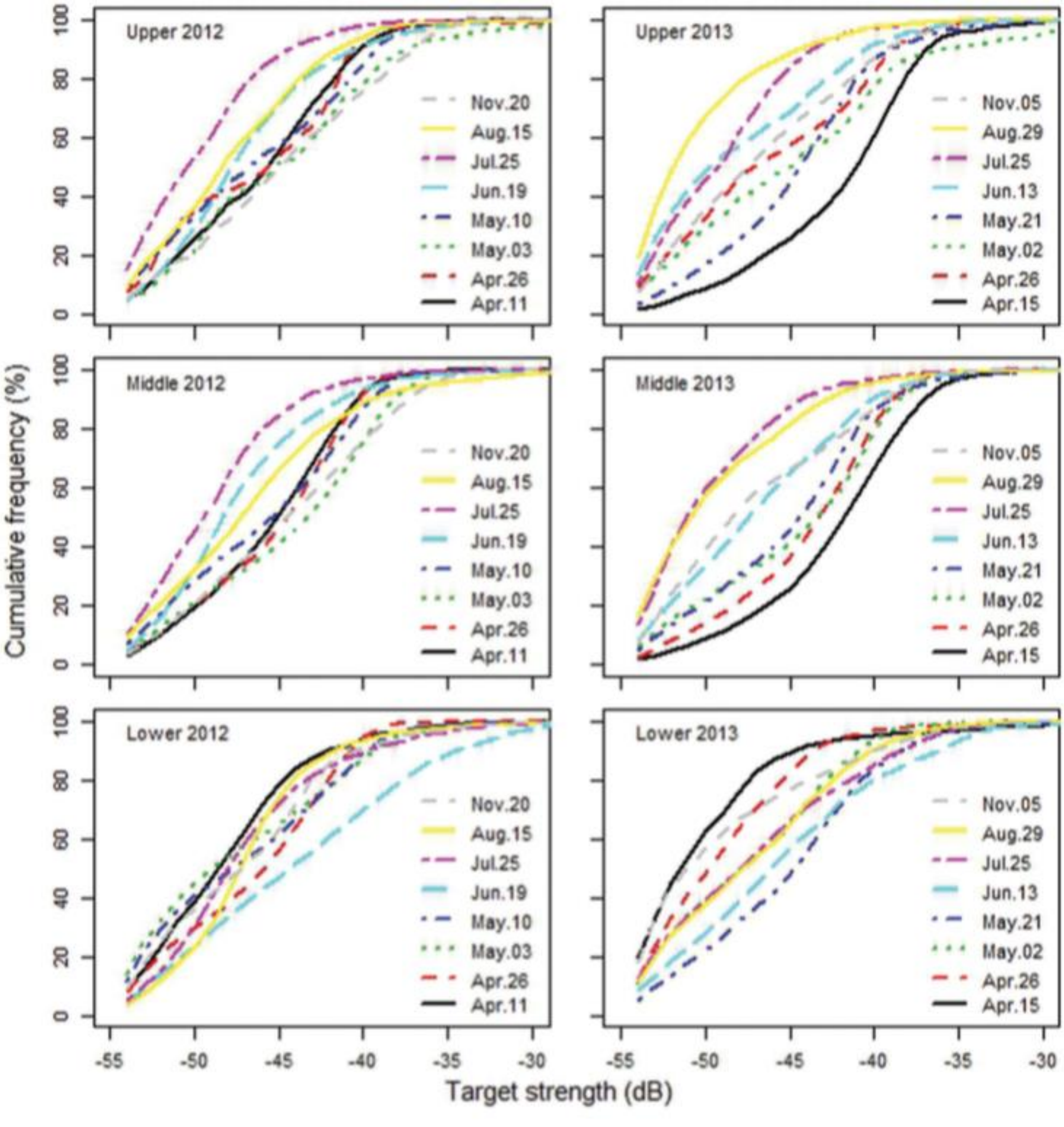


FIGURE 9. Cumulative frequency distributions of maximum TS per fish track (38 kHz) per stratum for transects conducted in the upper, middle, and lower estuaries in 2012 and 2013.

It was not possible to include the TS from fish tracks within detected fish schools in our analysis because it is difficult to resolve echoes when there are multiple fish ensonified in the beam. Therefore, the TS size-distribution plots we presented are biased towards discrete fish targets (fish deemed not to be in schools) and are not a true reflection of the size distribution of total fish present, particularly during surveys when schools were observed (i.e., May through November). Although we suspect that most schooling fish we detected were small clupeids, it is also likely that some schools contained higher TS fish (>−42 dB), including adult *Alosa spp*. and other larger adult diadromous species. For instance, it is possible that schooling fish present in the lower estuary in November are migrating diadromous fish (e.g., Rainbow Smelt and *Alosa spp*.). We observed fish aggregating loosely together in general areas in the middle estuary in April and May, presumably prior to upstream migration (e.g., Figure 2a); aggregating behavior in other parts of the estuary in varying hydrographic conditions may be different and species-specific. The parameters we set for fish schools were not designed for the expected response of specific species present in the estuary. Therefore, the distinction between fish aggregating close together and schooling requires future development. There is little known about the conspecific migratory behavior of diadromous species as they pass through estuaries, and therefore more work in this area is needed to further validate acoustic data.

Overall, we observed relatively few high-TS fish (i.e., >30 cm) throughout the survey. Fish tracks with the highest TS (significantly >−42 dB) in the Penobscot estuary would most likely be from large adult diadromous fish passing through the estuary during spawning, postspawning, or feeding migrations (e.g., Atlantic Salmon, American Shad, Striped Bass *Morone saxatilis*, Atlantic Sturgeon *Acipenser oxyrinchus*, Shortnose Sturgeon *A. brevirostrum*). The relationship of TS to TL varies depending on species, and the identity of the species acoustically detected in the estuary requires further validation. The generalized relationship of TS to TL for clupeids used here (Foote 1987) is unlikely to hold true for larger fish; however, it is encouraging that this survey detected fish tracks with high TS in the system. The relative scarcity in detection of fish with high TS overall is consistent with expectations given the presently low abundance levels of larger species specifically in the Penobscot estuary (Saunders et al. 2006; MDMR and MDIFW 2009). In the lower estuary, the higher TS fish tracks may be from diadromous fish as we surmised for the upper and middle estuaries; however, they may also be marine fish. It is not possible at this stage to definitively partition the acoustic fish tracks to species or even guild (e.g., diadromous, estuary resident, or marine).

The variability of environmental conditions and fish habitat use in this system required careful attention to spatial scale in survey design. For this survey the degree of coverage (Aglen 1989) was estimated at 10.5. This is a common measure of precision for acoustic surveys (Simmonds and MacLennan 2005) and a proxy for the coefficient of variation ($\mathrm{Cv_{proxy}}$) for the survey results. Our coverage results in a $\mathrm{Cv_{proxy}}$ of ~15% ($\mathrm{Cv_{proxy}}$ = 0.5/√degree of coverage), which is within the acceptable CV range for acoustic surveys. The spatial heterogeneity of fish observed in this study is likely a true description of habitat use and therefore a result of fish tolerance to the localized physical conditions, particularly salinity and life stage, rather than uncertainty in the survey design. In this survey, salinity appears to be an important variable in fish distribution. Whether the tide is ebbing or flowing may exert an unknown influence on the distribution of fish. Therefore, the direction of survey travel (transects upstream or downstream) may also influence our ability to accurately describe the spatial distribution of fish in the estuary at any given time. However, we standardized the survey by sampling at a consistent tidal height within a given location (the upper, middle, and lower estuary strata were consistently surveyed during high, mid, and low tide respectively, regardless of direction of travel). This was achieved by conducting the survey consistently in the direction of tidal flow (upstream transect direction on the flood tide and downstream transect direction on the ebb tide). The complexity of the salinity regime in the estuary is further influenced by variations in freshwater flow. We have shown that the location of the salinity mixing zone was variable, presumably influencing fish distributions. We predict that the distribution of fish is likely affected most by salinity conditions, and therefore the accurate delineation of strata boundaries based on salinity should be a concern during survey design. In this survey, the strata were fixed, based on expected general conditions, but in the future it may be more accurate to develop variable strata specific to each survey day, based on precise water column measurements of salinity.

The dynamic nature of the physical environment in estuaries affects a variety of acoustic propagation variables, which in turn may affect fisheries acoustic measurements. Speed of sound and attenuation are two variables that are affected by changes in temperature and salinity, which directly influence calculations of acoustic wavelength and attenuation, which in turn affect calculations of range (e.g., seabed depth) and sampling volume (e.g., beam angle). These effects can ultimately influence estimates of target strength as well as volume and areal density. Salinity typically has the greatest range of values, of which salinities of near 0‰ occur in the upper estuary and salinities near that of oceanic water(e.g.,34‰) occur in the lower estuary. Temperature and salinity of ambient conditions were used for echo-sounder calibrations, but the median temperature and salinity for the entire range of the survey were used to process the acoustic data. This choice essentially standardized the measurements to the medium conditions measured over the course of the survey area and had minimal impact on the measurements. For example, at 8°C, 10-m depth, and salinities of 0, 17, and 34‰, the speed of sound was calculated to be 1,439.3, 1,460.4, and 1,481.4m/s, respectively (Fofonoff and Millard

1983). Usingthe median salinity value resulted in a maximum of 1.4% error in range calculations. The maximum depth encountered during our surveys was 40 m, giving a maximum error of ~0.5 m in the lower estuary, but with typical water depths in the upper estuary of~15 m, an error of ~0.2m. At 38kHz, 8°C, and salinities of 0, 17, and 34‰, attenuation was calculated to be 0.49, 5.28, and 10.09 dB/km, respectively (Medwin and Clay 1998). At 40 m range and using the median salinity, a maximum error of 0.2 dB is expected at the far ends (i.e.,river mouth and farthest up stream extent) of the survey. The total angular beam width for the 38kHz transducer (nominal beam width of 7°) for 8° Candsalinities of 0, 17, and 34‰, were 6.7, 6.8, and 6.9°, respectively (D. Chu, Northwest Fisheries Science Center, personal communication). These translate to about a 1.5% change in sampling volume at opposite ends of the survey. We conclude that this is an acceptable level of sampling volume error considering the physical variability in the system.

Our results provide evidence of identifiable spatial, temporal, and size-distribution patterns of fish occurring in the estuary. Allocation of species to the acoustic backscatter of aggregations or schools appears to be an achievable goal using appropriate fish capture validation methods (e.g., Stables et al. 2005; ICES 2015b). The first step in the classification of acoustic data to species is to identify behavioral patterns based on prior knowledge of the potential species' life histories. These behavioral patterns can be used to develop echogram scrutiny protocols based on observed distribution patterns (i.e., size-dependent spatial aggregations, layering, and dense schooling and seasonal or salinity patterns). Species identification and size distribution of species from a trawl or other survey methods (e.g., high-frequency, acousticimaging sonar) could allow abundance and biomass estimation per species and area. A combination of validation methods would be beneficial because of the dynamics of the system, the species known to inhabit the system, and the different aggregating behaviors of fish observed in this study. Thus, the development of measurable indices from data collected from hydroacoustic surveys can be used to monitor changes in pelagic fish abundance and biomass in the estuary over time.

In conclusion, we described spatial and temporal patterns of $s_A$ and TS in the Penobscot estuary over the course of 2 years with relatively low effort using acoustic methods. Describing relative acoustic measures of $s_A$ and TS in a dynamic system of this size in a relatively short time (~5-h transect) is a positive development. The methods described here can be replicated and with further development of species validation methods can provide a template for long-term multispecies monitoring and hypothesis testing of pelagic fish density (from $s_A$) and fish size (from TS) distributions in temperate estuaries of similar size and depth. Estuaries are inherently variable, but measuring fish distribution patterns within defined spatial and temporal strata appears to be a realistic objective in these dynamic ecosystems.

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